SHORT COMMUNICATION

Deletion Analysis of Essential Genes of Escherichia coli: Investigation of the btuB-rpoBC Interval

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A method is described for deletion mapping of essential genes in Escherichia coli. It involves the isolation of secondary-site insertions of λcI857plac5 into an F' plasmid. The transposed lacZ gene is useful both for the ready screening of plasmid–phage cointegrates and for rapid analysis of deletions that extend from the site of phage integration into the bacterial genes carried on the substituted plasmid. Such deletions may be used to 'hook-up' bacterial cistrons to the powerful lac promoter. We report the application of this technique to the study of the btuB–rpoBC interval, a cluster of genes encoding components of the transcription–translation apparatus.

INTRODUCTION

The isolation of transducing phages carrying bacterial sequences, and the use of cloning procedures have proved extremely useful for the deletion analysis of indispensable cistrons (see, for example, Fiil et al., 1980; Newman & Hayward, 1980; Nomura & Post, 1979; Scaife et al., 1980). Our approach to deletion analysis of essential genetic material has been to obtain secondary-site insertions of λcI857plac5 in an F' plasmid. The isolation of F' plasmid–λcI857plac5 cointegrates is made feasible by the ease of screening extra-chromosomal material. Moreover, the presence of the prophage lacZ gene both circumvents the necessity of selecting for insertional inactivation and aids the determination of deletion endpoints. We report the application of this method to the btuB–rpoBC interval (89.1 to 89.5 min), a region rich in genes encoding components of the transcription–translation apparatus (Bachmann & Low, 1980).

METHODS

Bacterial and phage strains. The starting strain for the isolation of plasmid–λ cointegrates, AJ249, was constructed from AJ217, a spontaneous Mal+ derivative of AJ7 [argG metB lacZ53(Am) mal(λ-r) recA1 rpsL(Str-r) rpoB70(Rif-r)] (Boyd et al., 1974). The following markers were first introduced into AJ217 by conjugation: Δ(gal-att- bio) from KS302 [thi Δ(gal-att-bio) HfrH (Shimada et al., 1972)]; Δ(lac-pro)XIII from CA9005 [thi Δ(proAB-lac)XIII tsx HfrH]; and recA56 from JC5088 (ile thr recA56 spc Hfr KL16). A spontaneous phage BF23- and colicin E3-resistant derivative (btuB) was then obtained. Finally, the F' plasmid, F110, carrying the metB-malB region, was introduced into AJ39 (AJ7/F110). λ amber derivatives were kindly supplied by M. E. Gottesman and Noreen Murray; λcI90c17 was obtained from R. Buxton.

Media and general methods. Minimal media were prepared with Meynell salts (Meynell & Meynell, 1970) supplemented with 0.2% (w/v) glucose or lactose, and amino acids at 20 μg ml⁻¹. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (40 pg ml⁻¹) and isopropyl-β-D-thiogalactopyranoside (10⁻³ M) were included in Lac indicator plates. Where necessary, rifampicin was added to a final concentration of 200 μg ml⁻¹. Complex medium was 2.5% Oxoid nutrient broth no. 2; this was supplemented for phage λ work with 0.01 M-MgSO₄ and 0.2% sucrose. Phage lysates were prepared by the standard plate technique (Miller, 1972). Colicin E3 extracts were obtained by a salt extraction procedure (Hunter & Glass, 1982).

Isolation of cointegrates of F110 and λcI857plac5. Starved AJ249 cells were treated with first λcI857plac5 and then λk80c (Shimada et al., 1972). Lysogens were isolated on minimal glucose plates at 30 °C. They were screened...
for resistance to phage BF23 (and colicin E3) and rifampicin by dilution replica-plating to avoid false positives (Hunter & Glass, 1982). Met+ transconjugants of AJ252 (AJ7 btuB lacI857) were tested for resistance against phage BF23/colicin E3 and rifampicin. Positive clones were checked for their ability to produce β-galactosidase on minimal Lac indicator plates. In no case was direct selection used to obtain cointegrates: putative btuB::λ and rpoB::λ insertions were obtained from the original lysogen plate.

Properties of cointegrates. Spontaneous and heat-pulse curing frequencies and prophage number were determined as described by Shimada et al. (1972). Revertants that carry a functional rpoB or btuB allele on the F+ were selected from among a population of Ts+ Rec- survivors. rpoB+ revertants were selected in AJ5022 [AJ7 rpoB12(Am)F100-13 supE (Nene & Glass, 1981)] on minimal Lac indicator plates; transconjugants of AJ5022 that carry F110rpoB+ should be Lac−. In the case of the btuB::λ insertions, a metE btuB recA recipient [KL3000, leu-6 metE70 proC32 lacI3 lacZ18(Oc) malA(λr) ara-14 mil-1 xyl-5 thi-1 recA1 str-109 spe-15 gyrA btuB supD (Birge & Low, 1974)] was employed since a functional btuB allele is necessary to enable the auxotroph to utilize exogenous vitamin B12(Am)F100-13.

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RESULTS AND DISCUSSION

In the absence of a functional attachment site (ΔattP), phage λ integrates at secondary sites that show some homology with the normal region (Shimada et al., 1972, 1973). We have used this property to obtain stable secondary-site lysogens in which the prophage is present on an F+ plasmid rather than the chromosome. The substituted plasmid employed in this study, F110, spans the metB–malB region (about 105 Mdal, data not shown).

Cointegrates of F110 and λcl857plac5 were identified from among homo-immune lysogens capable of complementing a metB Δlac λ+ recipient (AJ252) in a genetic cross. [Zygotic induction, the killing of non-lysogenic recipients upon λ transfer, is not required for detecting the F110::λ insertions.] They were screened for insertion at btuB and rpoB, the genes for the B12 multivalent receptor and the β subunit of RNA polymerase. Insertional inactivation of btuB+ (in a btuB+/btuB− merodiploid) renders the secondary lysogen resistant to phage BF23 and E coli colicins (and unable to make use of the B12-dependent homocysteine transmethylase: Jasper et al., 1972, Kadner & Liggins, 1973). Similarly, integration of λcl857plac5 into the dominant, rifampicin-sensitive allele, rpoB+, on F110 allows growth in the presence of the antibiotic.

We have obtained a number of independent insertions at btuB and rpoB; their respective position is supported by deletion analysis (see below). The division of these lysogens into three main types according to their properties (Table 1) suggests that there are at least two secondary sites for λcl857plac5 in btuB: only a single class of rpoB insertions was found. Krueger et al. (1981) have recently reported that there are several insertion sites for λcl857 in the btuB region.

Starting with two sets of cointegrates, btuB::λcl857plac5 and rpoB::λcl857plac5, we have obtained a large number of different deletions that span the btuB–rpoBC region. This is made possible by the presence of the thermolabile phage repressor (the cl857 gene product) and the fact that survivors carrying the recA56 allele invariably lack both prophage and bacterial material (see Table 1). Loss of bacterial markers is readily ascertained by transfer of the F′ deletion derivatives to KA2005 [argE metB thr trp btuB lac rpoB(Rif-r) purH/D recA (Armstrong & Herman, 1976)]; deletions of prophage material were detected by making use of λ amber derivatives (Shimada et al., 1972).

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Table 1. Properties of secondary-site λCl857plac5 lysogens

<table>
<thead>
<tr>
<th>Insertion site*</th>
<th>Plasmid class†</th>
<th>Curing frequency‡</th>
<th>Burst size</th>
<th>λCl90c17 test§</th>
</tr>
</thead>
<tbody>
<tr>
<td>F110 btuB</td>
<td>pQN10</td>
<td>4.6 × 10⁻⁶</td>
<td>2.4 × 10⁻³</td>
<td>0.001 sensitive</td>
</tr>
<tr>
<td>pQN11</td>
<td>2.3 × 10⁻⁵</td>
<td>2.4 × 10⁻³</td>
<td>69</td>
<td>sensitive</td>
</tr>
<tr>
<td>F110 rpoB</td>
<td>pQN22</td>
<td>6.8 × 10⁻⁷</td>
<td>1.9 × 10⁻²</td>
<td>0.4 sensitive</td>
</tr>
</tbody>
</table>

* A total of 10835 secondary-site lysogens (of AJ249) were obtained at a frequency of 4.6 × 10⁻³. Of these, 0.028% carried the phage integrated into rpoB on F110 and 1.72% (of 1048 lysogens screened) carried insertions into btuB.
† The properties of strains carrying F110 btuB : : λCl857plac5 and F110 rpoB : : λCl857plac5 (burst size, λCl90c17 sensitivity, and deletion profiles; Table 2) suggest that there are three main insertion sites, two in btuB and one in rpoB. Results for representative plasmid derivatives are given.
‡ Although heat-pulse curing is an effective method for obtaining λ-derivatives, curing rarely stems from exact excision in a recA background. We have been unable to isolate revertants encoding the wild-type function, despite positive selection (btuB+ is regenerated in a recA+ strain). Thus, all Ts+ survivors obtained in a recA background carry extensive deletions (Table 2). This is consistent with the observation that most recA mutants do not support spontaneous induction (Roberts & Roberts, 1981).
§ In early studies on recA+ strains (Shimada et al., 1972), there appeared to be two classes of secondary lysogens: those giving burst sizes > 100 (putative polylysogens), and those with a burst size ≤ 0.1 (possible single lysogens). [In the case of single insertions into the R plasmid, R100, burst sizes were near to, or less than unity (Dempsey & Willetts, 1976).] Although burst-size data suggest that the F110 : : λCl857plac5 cointegrates, pQN11 and pQN22, are multiple (tandem) lysogens, we have been unable to substantiate polylysogeny using λCl90c17, a phage that can plate upon single but not multiple lysogens. The lack of correlation between burst size and λCl90c17 sensitivity presumably reflects the low copy number of F110 (the wild-type F' appears to be present in about 0.5 copies per chromosome: Hayward et al., 1974); copy number may decrease with plasmid size (Pritchard, 1978).

Table 2. Deletion profile for btuB : : λCl857plac5 and rpoB : : λCl857plac5 secondary-site insertions

<table>
<thead>
<tr>
<th>Proportion of survivors*</th>
<th>Extent of deletion</th>
<th>btuB insertion</th>
<th>rpoB insertion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pQN10</td>
<td>pQN11</td>
<td>pQN20</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>HP</td>
<td>S</td>
</tr>
<tr>
<td>arg</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>btuB</td>
<td>0.2</td>
<td>9.5</td>
<td>4</td>
</tr>
<tr>
<td>lac</td>
<td>0</td>
<td>0</td>
<td>84</td>
</tr>
<tr>
<td>λci</td>
<td>0</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>rpoB</td>
<td>0</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>purD</td>
<td>0</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.4</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>37.8</td>
<td>37.8</td>
</tr>
</tbody>
</table>

* The two types of secondary-site insertions in btuB (see Table 1) are represented by plasmids pQN10 and pQN11; a total of 1125 spontaneously occurring deletions (430 for pQN10 and 695 for pQN11), and 1117 heat-pulse induced deletions (897 for pQN10 and 220 for pQN11) have been analysed. In the case of the rpoB insertion, a total of 643 spontaneously occurring deletions and 1324 heat-pulse induced deletions have been studied. For both btuB and rpoB secondary-site lysogens, heat-pulse induced deletions were consistently shorter. S, Spontaneously occurring; HP, heat-pulse induced.
† pQN20 deletions that remove λc1 and purD should result in loss of the promoter-distal portion of rpoB: this was not tested for.

would be expected (Pfeifer et al., 1974), to arise from specific processes. In the case of spontaneously generated deletions, there appears to be a 'hotspot' beyond purD since the majority of deletions have one endpoint that removes this locus (see also Linn et al., 1979). The large number of deletions isolated are useful for fine structure analysis of the transcription—translation gene
cluster at 89 min. They can, for example, be transferred to $\lambda rpoB$ transducing phage (for instance, $\lambda dtf^{18}$: Kirschbaum & Konrad, 1973) as described by Linn et al. (1979). Furthermore, the insertion of lacPoz adjacent to (or within) $rpoB$ is a potential starting point for the in vivo construction of fusions placing transcription–translation genes under lac control (and vice versa).

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REFERENCES


